RESEARCH NOTE

Evolution, antimicrobial susceptibility and assignment to international clones of methicillin-resistant *Staphylococcus aureus* isolated over a 9-year period in two Spanish hospitals

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ABSTRACT

Antimicrobial resistance profiles, restriction fragment length polymorphism of the coagulase gene and repetitive element sequence-based PCR were used to classify 210 methicillin-resistant *Staphylococcus aureus* isolates recovered between 1997 and 2005 in two hospitals in Vigo, north-west Spain. Representative isolates belonging to the epidemic clones were analysed by *spa* typing and multilocus sequence typing, and the staphylococcal chromosomal cassette (SCC)*mec* type was determined for all isolates. The New York/Japan clone (t002-ST5-II) was detected in Spain for the first time. However, the New York/Japan and the Brazilian (t037-ST239-IIIA) clones were replaced by EMRSA-16 (t018-ST36-II), which at present is the predominant clone.

Keywords Epidemic clones, identification, molecular analysis, MRSA, Spain, typing

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The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) is higher in southern than in northern Europe [1]. According to a national multicentre study, the incidence of MRSA in Spain has increased progressively [2]. During the 1980s, the Iberian clone spread throughout the country [3], although subsequent studies have demonstrated the replacement of this epidemic clone [4–6]. The aim of the present study was to identify the epidemic MRSA (EMRSA) clones in two hospitals in Vigo, north-west Spain, during the 9-year period 1997–2005. In total, 210 MRSA isolates were obtained from individual patients: 82 (39%) in internal medicine wards; 71 (33.8%) in intensive care units; 23 (11%) in trauma units; and 34 (16.2%) in other wards. The most frequent sites of isolation of MRSA were wounds (37.5%), sputum (36.6%) and intravenous catheters (11%). The frequency of MRSA in Hospital I was 28% in 1998, rising to 43% in 1999, and then decreasing to 3% in 2005, while in Hospital II the frequency increased progressively from 16% in 1997 to 22.3% in 2005. An isolate was defined as an EMRSA strain on the basis of the definition of Hoefnagels-Schuermans *et al*. [7].

Susceptibility testing was performed by the standard disk-diffusion method according to CLSI guidelines [8]. Spectinomycin susceptibility was determined as described previously [9]. The isolates were confirmed as MRSA by amplification of the *mecA* gene [10]. *S. aureus* ATCC strains 25923, BAA-40, BAA-41, BAA-43 and BAA-44 were included for quality control purposes. All isolates were screened for mupirocin resistance by the disk-diffusion method [11]; MICs were determined for isolates identified as resistant, and the presence of the *mupA* gene was determined by PCR [12]. In total, 45 isolates (15 isolates of each epidemic clone) were tested by the vancomycin agar screen method to detect heterogeneously vancomycin-resistant *S. aureus* or intermediately vancomycin-resistant *S. aureus* [13].

Analysis of restriction fragment length polymorphism of the coagulase gene (PCR-RFLP *coa*) and repetitive element sequence-based PCR (rep-PCR) were performed as described previously [14,15]. Patterns differing by one or more bands were categorised as distinct clonal types. The staphylococcal chromosome cassette (SCC)*mec* structural types were determined by a multiplex PCR strategy [16]. Randomly selected MRSA isolates belonging to the epidemic clones were further studied by *spa* typing (24 isolates) and multilocus sequence typing (six isolates) [17,18].

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Table 1. Phenotypic and genotypic characteristics of the three major epidemic clones

Genotype: no. of isolates (%)	Resistance profile: no. of isolates (%)	SCC*mec* type (%)	*spaA* type (no. of isolates tested)	Sequence type (ST) (no. of isolates tested)	Clone name
A: 76 (36.2)	1: 76 (36.2)[a]	IIIA (36.2)	t037 (8)	ST239 (2)	Brazilian
B: 25 (11.9)	2: 22 (10.5)[b]	II (11.9)[d]	t002 (8)	ST5 (2)	New York/Japan
C: 80 (38.1)	3: 80 (38.1)[c]	II (38.1)	t018 (8)	ST36 (2)	EMRSA-16

[a](GEN, TOB, CIP, CLI, ERY, SXT, CH)^R; (RIF, TET, NIT, SPT)^S.
[b](CIP, CLI, ERY, SPT)^R; (GEN, TOB, SXT, RIF, TET, NIT,CH)^S.
[c](TOB, CIP, CLI, ERY, SPT)^R; (GEN, SXT, TET, NIT, CH)^S (variable susceptibility to RIF).
[d]The band corresponding to the pUB110 insertion was not observed.
GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; CLIN, clindamycin; ERY, erythromycin; SXT, trimethoprim–sulphamethoxazole; RIF, rifampicin; TET, tetracycline; NIT, nitrofurantoin; SPT, spectinomycin; CH, chloramphenicol.

The majority (84.8%) of MRSA isolates belonged to one of three resistance profiles, based on a panel of 11 antimicrobial agents (Table 1). None of the 45 isolates screened was intermediately vancomycin-resistant *S. aureus* or heterogeneously vancomycin-resistant *S. aureus*. Nine isolates were resistant to mupirocin by the disk-diffusion method, and three (1.4%) of these showed high-level mupirocin resistance (>256 mg/L). The *mupA* gene was only detected in these three isolates, of which two were the UK EMRSA-16 strain.

PCR-RFLP *coa*, rep-PCR and epidemiological data clustered the majority (86.2%) of the isolates into three EMRSA clonal types (Table 1). The remaining isolates (13.8%) were classified as sporadic. Overall, 95% of the isolates were hospital-acquired and 5% were associated with other healthcare facilities; no community MRSA strain was isolated.

The results of SCC*mec* typing, *spa* typing and multilocus sequence typing are summarised in Table 1. Fig. 1 illustrates the temporal evolution of the three EMRSA clones.

Typing is one of the most important steps involved in understanding and controlling MRSA infections. Pulsed-field gel electrophoresis analysis, although technically demanding, is considered to be the reference standard typing method [14]. However, the present study used PCR-RFLP *coa* and rep-PCR because of their accessibility for laboratories with limited resources. Moreover, these methods have been shown to have a good correlation with pulsed-field gel electrophoresis analysis [7,14]. These PCR-based methods, in conjunction with the epidemiological data, grouped the isolates into three main epidemic clones. Antimicrobial resistance patterns allowed a clear distinction among the three epidemic

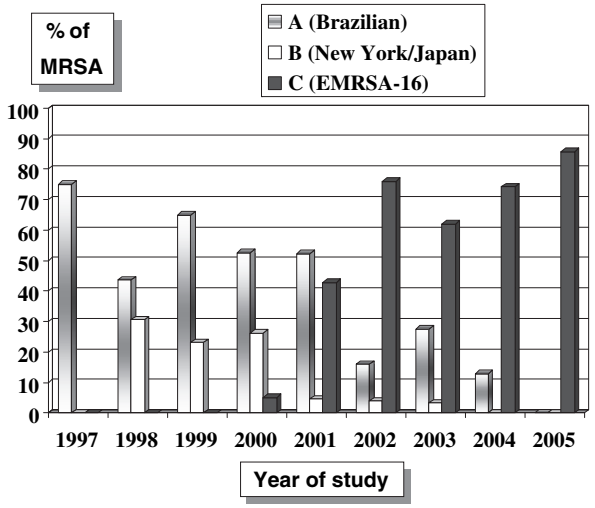

Fig. 1. Temporal evolution of the three epidemic methicillin-resistant *Staphylococcus aureus* (MRSA) clones between 1997 and 2005 at the two hospitals included in the study.

clones and sporadic isolates, as has been reported previously [7]. Rapid classification of an isolate as epidemic or sporadic could be very useful in allowing control measures to be focused on the most important strains, thereby leading to a substantial decrease in the incidence of MRSA and the financial and logistical consequences [19].

The limited number of isolates analysed in the present study by *spa* typing and multilocus sequence typing could add some bias to the results. Nevertheless, the homogeneity in the antimicrobial resistance patterns and the results of genotyping support the decision to randomly analyse representative strains of the epidemic clones. The Iberian clone, widespread in Spain before 1996, was not detected [3,5], and the Brazilian clone predominated until 2002. The latter clone could have spread to Vigo from Portugal, where it displaced the Iberian clone during the 1990s [9]. The subsequent replacement of the multiresistant Brazilian clone by the pandemic EMRSA-16 clone suggests that EMRSA-16 could become the prevalent clone in Spanish hospitals [4,6]. The EMRSA-16 clone identified in the present study was susceptible to gentamicin but resistant to tobramycin; this phenotype has become common in French hospitals [20]. The reason(s) for the emergence and rapid spread of these strains remains unclear.

To our knowledge, this is the first report of the isolation of the New York/Japan clone in Spain. Interestingly, the plasmid pUB110 was absent from the New York/Japan isolates, which were

susceptible to gentamicin and tobramycin [16]. A similar observation has been made in Asia [21].

As in other European studies [22], no heterogeneously vancomycin-resistant *S. aureus* or intermediately vancomycin-resistant *S. aureus* isolates were identified. Nevertheless, it is necessary to continue surveillance for these phenotypes, as the use of glycopeptides continues to be high. Despite the increase in mupirocin resistance among MRSA in Spain [11], resistance to mupirocin remained at a low level (1.4%) in the present study. However, two MRSA isolates with high-level mupirocin resistance were identified as belonging to the UK EMRSA-16 clone. As the capacity of this clone to acquire resistance to mupirocin is unknown, there remains the possibility of the emergence of MRSA strains highly resistant to mupirocin in the future.

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